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AVIAN BOTULISM (TYPE A) OR LIMBER NECK

ROBERT GRAHAM AND HERMAN SCHWARZE

From the Department of Animal Husbandry, Laboratory of Animal Pathology, University of Illinois, Urbana

A sporadic toxemic syndrome in chickens, or a closely allied disease of the barnyard fowl, has been recognized for many years in the urban and rural poultry flocks of America. Affected birds display such prominent symptoms as weakness, incoordination of movement, prostration, coma, and inappetence, while the striking feature of the disease is a weak, pendulant, or limber neck. The latter, easily recognized, symptom has been popularly designated as the name of the disease.

Until Dickson¹ suggested that *B. botulinus* might be an etiologic factor in so-called limber neck, little was conjectured relative to the cause or causes of this disease in poultry. In his investigation of a case of botulism in man, a fatal illness in chickens developed simultaneously. The clinical manifestations in the fatally afflicted chickens were repeatedly induced by feeding portions of contaminated human food, and *B. botulinus* was isolated from the alimentary tract of affected birds. Since this observation, investigators have expressed a deep interest in the etiology of limber neck. The toxic effect of *B. botulinus* when fed to chickens has been sought as a contributing or supporting factor of Dickson's original contention, but the results in the hands of independent investigators are not in full agreement.

TOXIC CHARACTER OF *B. BOTULINUS* FOR CHICKENS DEPENDENT ON TYPE OF TOXIN

It is even possible that the symptoms characteristic of limber neck may involve a group of diseases induced by a variety of agents, yet it seems well established from the evidence at hand that one type of the syndrome under discussion may be induced by *B. botulinus* type A; while *B. botulinus* type B as yet has not been found associated with the sporadic poultry disease in question. Moreover, in repeated trials it has not been possible to produce illness in chickens by feeding liberal amounts of *B. botulinus* type B disguised in wholesome feed.

The numerous discrepancies reported relative to the toxicity of *B. botulinus* for chickens is responsible for the opinion that this character

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¹ Monograph No. 8, Rockefeller Inst. for Med. Research, 1918.

may not be an attribute of all strains of the organisms or that the susceptibility of the exposed birds or the quantity of toxin consumed may be an important or deciding factor. While such relative factors cannot be entirely excluded, the incidence of illness in chickens following the feeding of the proper amount of specific botulinus toxin apparently depends on the type of the toxin employed, in accord with the suggested biochemic classification of Burke. The elimination of misunderstanding or confusion on this point will, therefore, doubtless imply more attention to a specific designation of the strain of *B. botulinus* employed. Recognition of each strain of *B. botulinus*, referred to in literature in accord with the present arbitrary classification as types A and B, is dependent, in our opinion, on the expressed toxicity of the strain for chickens.

It is not intended to refute the possible relation of the many specific or nonspecific factors that have been mentioned by investigators, clinicians and poultrymen in connection with this disease in poultry, but to restrict and qualify, if possible, in accord with our findings, the seeming etiologic relation existing between limber neck in chickens and avian botulism with *B. botulinus* type A. The outbreaks of limber neck in poultry coming to our attention have not been associated with illness in the human family as reported by Dickson. Nor have the losses, to our knowledge, occurred on such an extensive scale as reported by Hart,² yet the economic importance of diseases of poultry similar to limber neck has been repeatedly suggested by the mortality in several small flocks.

CHICKENS REFRACTORY TO *B. BOTULINUS* TYPE B

Following the report by Dickson³ that chickens were susceptible to *B. botulinus* and that the clinical manifestation resembled so-called limber neck, Buckley and Shippen⁴ fed chickens *B. botulinus* isolated from cheese without producing noticeable symptoms, while Wilkins and Dutcher⁵ concluded, "It was not possible to produce limber neck symptoms in poultry by feeding and injecting the toxins produced by three different strains of *B. botulinus*. The strains were toxic, however, for guinea-pigs." Previous to either report, Van Ermengem and Roemer found that chickens were not susceptible to certain strains of *B. botulinus*. We repeatedly attempted to determine the toxicity of botulinus

² Jour. Amer. Vet. Med. Assn., 1920, 10, p. 75.

³ Ibid., 1917, 3, p. 612.

⁴ Ibid., 1917, 3, p. 809.

⁵ Ibid., 1920, 10, p. 653.

toxin to chickens by feeding liberal amounts of the toxin. From 2 to 4 mature chickens were included in each lot. The toxin was carefully mixed in the feed or given from a capillary pipet. Only negative results were obtained. Since these first results were reported,⁶ eight strains of *B. botulinus*, all of which later proved to be type B, isolated from remote outbreaks in food poisoning in domestic animals, have been fed to mature chickens. Symptoms of illness could not be detected at any time in these fowls. The negative results reported in feeding *B. botulinus* to chickens suggest, in the light of our present knowledge, that other investigators also employed type B strains in their experiments.

THE RELATION OF CHICKENS OR OTHER RESISTANT SPECIES
TO THE EPIZOOLOGY OF BOTULISM

Although the barnyard fowl and other animals may possess a natural tolerance to one or the other type of botulinus toxin, it cannot safely be assumed that they may not be an important factor in the distribution of the virus. Mature swine have displayed a marked resistance to *B. botulinus* type A, while under certain conditions mature cattle have apparently possessed a marked tolerance, though the virus could be found in the excreta of these animals. The latter observations have been repeated when unfiltered broth cultures of *B. botulinus* have been fed to hyperimmune horses, i. e., the feces have been found to contain the spores of the organism. Buckley and Shippen⁷ observed that chickens fed *B. botulinus* toxin remained healthy, yet the feces of the fowls proved poisonous to horses when disguised in their feed. In one instance poisonous chicken feces were found in an oat hay⁶ which had been given horses, among which a sporadic outbreak of botulism had occurred. It is significant that the farm poultry on the premise remained healthy, and that these birds had limited access to other feeds stored in the barn. The relation of the presence of *B. botulinus* (later proved to be type B) to the chicken excreta in the feed in question was not definitely established, yet it was suggested that one of the following possibilities might be true:

- (a) The chicken excreta had primarily contaminated the oat hay.
- (b) The chickens had eaten of the contaminated oat hay and the virus had passed through the avian alimentary canal to the forage unaltered, or if altered en route, had regained its virulence.

⁶ Graham, Robert; Brueckner, A. L., and Pontius, R. L.: Ky. Agric. Exper. Sta. Bull. 207, 1917, p. 60.

⁷ Jour. Amer. Vet. Med. Assn., 1917, 3, p. 809.

(c) The chicken excreta had been discharged uncontaminated with *B. botulinus*, and by direct contact with the unwholesome oat hay had become contaminated with *B. botulinus*.

B. BOTULINUS TYPE A FATAL TO CHICKENS

The toxic effect of *B. botulinus* type A on chickens was first observed in feeding a strain isolated from olives. Typical symptoms of limber neck could be repeatedly induced by adding the toxin to the feed or by giving it with a capillary pipet. The striking variation in the pathogenicity of different strains of *B. botulinus* to chickens suggested the importance of classifying the toxins according to the method of Burke.⁸ All strains that failed to induce illness in chickens were found to be *B. botulinus* type B, while two strains from olives, one strain associated with a fatal pasture disease of horses, one strain from the feed of swine associated with the death of nursing pigs, one strain from the spleen of a calf, as well as other strains from outbreaks of limber neck in poultry, which on being fed to chickens produced fatal illness indistinguishable from limber neck, proved to be *B. botulinus* type A. The symptoms may be observed from 5 to 6 hours after the feeding of the toxin and death may follow in 24 to 48 hours.

The evidence strongly suggests that an untyped strain of *B. botulinus* may be readily and accurately recognized by feeding the toxin to chickens. As an independent, confirming or preliminary method of identifying or typing *B. botulinus*, it may be employed to expedite a diagnosis without incurring the delay involved in isolating and propagating the specific anaerobe, which may involve several days or weeks. This preliminary determination may be accurately accomplished if unaltered contaminated food is available for feeding purposes. Furthermore, should a careful survey in outbreaks of botulism in domestic animals reveal the remote or even simultaneous occurrence of limber neck in chickens, the writers would feel justified in recommending specific *B. botulinus* antitoxin type A. In fact, our experience leads us to believe that the latter antitoxin is indicated in the treatment of this disease in poultry in preference to the polyvalent variety.

AVIAN BOTULISM TYPE A

The concrete relation of *B. botulinus* Type A to the sporadic disease in poultry designated limber neck is briefly suggested in the bacteriologic findings of the following typical outbreak.

⁸ Jour. Bacteriol., 1919, 4, p. 555.

Specimens 823 to 829, delivered to the laboratory Oct. 22, 1920, consisted of 6 full-grown chickens. The birds were prostrate and unable to stand. According to the owner, the flock, which consisted of 70 birds, was apparently in good health until 3 days before. On this date one bird was found dead. The following day 2 more dead birds were found in the yard. The third day, and at the time the specimens were delivered, between 10 and 15 birds were visibly ill. On the same afternoon a survey of the premise and a careful inquiry relative to the feed given, revealed the fact that no grain was regularly fed to the flock. A limited amount of corn was probably obtained by the birds from a nearby corn field, as well as corn fodder stacked near the barn. The latter appeared wholesome and was being fed to a cow. At least a portion of the ration consumed by the chickens consisted of freshly gathered restaurant garbage which the birds obtained from the feeding floor of an adjoining pig lot. We gathered samples of this refuse from the feeding floor in sterile vials for examination. The pigs consuming the garbage remained healthy, yet the mortality in the fowls approximated 80%, with the losses distributed over a period of two weeks.

B. BOTULINUS TYPE A ENCOUNTERED IN GARBAGE AND INTESTINAL CONTENTS OF DEAD CHICKENS

Omitting the details of the examination, *B. botulinus* type A was encountered in the composite sample from the intestinal tract of several of the dead birds, as well as from a composite sample of the garbage of the feeding floor. The two strains isolated proved analogous and capable of inducing the syndrome of limber neck in chickens. The result of the typing test is shown in table 1.

TABLE 1
RESULTS IN TYPING TEST

Number	Weight of Chickens, Gm.	Treatment* Nov. 5, 1920, 10 a. m.	Results
424	250	100 L. D.† <i>B. botulinus</i> , strain 829, 10 units type A antitoxin.....	Remained healthy
449	260	100 L. D. <i>B. botulinus</i> , strain 829, 10 units type B antitoxin.....	
428	250	100 L. D. <i>B. botulinus</i> , no antitoxin.....	
			Dead, Nov. 6, 1920, 7 a. m.
			Dead, Nov. 6, 1920, 7 a. m.

* Toxin administered via the mouth with capillary pipet; antitoxin injected simultaneously beneath the skin.

† Guinea-pig lethal dose.

The A type antitoxin used in this test was a sample from Dr. George A. Hart, Veterinary Department, University of California. The history of the strain from which the antitoxin was produced is not known to us, yet the serologic relation to avian type A, strain 829, is clearly suggested.

BOTULINUS ANTITOXIN TYPE A, A SPECIFIC PROPHYLACTIC

Limber neck in chickens induced by *B. botulinus* type A may be prevented in healthy fowls by injections of 50 units, more or less, of specific type A antitoxin. The results of immunologic tests in chickens are suggestive of the value of antitoxin in the control of this condition in natural outbreaks. The curative value of the antitoxin in chickens

is unknown, since the few trials to which it has been subjected to date have not permitted definite deductions. As a prophylactic it has not, as yet, been used extensively under field conditions, but in the laboratory it has been repeatedly observed that 50 units of antitoxin are quite sufficient to protect mature chickens against 800 lethal doses of toxin for guinea-pigs. In fact, the latter amount of toxin may be repeated on three or four consecutive days without producing noticeable illness, if preceded by a subcutaneous injection of antitoxin.

The type B antitoxin is not in our observation indicated in the treatment of limber neck. Its failure to control the disease as induced by type A is shown in Table 2.

TABLE 2
RESULTS OF USE OF TYPES A AND B ANTITOXIN

Number	Weight Gm.	Toxin-Antitoxin Dec. 20, 1920, 10 a. m.	Results
869	1,430	829/800 L. D. 20 u. A.	Remained healthy
870	1,460	829/800 L. D. 40 u. A.	Remained healthy
871	1,570	829/800 L. D. 80 u. A.	Remained healthy
872	1,640	829/800 L. D. 160 u. A.	Remained healthy
873	1,600	829/800 L. D. 100 u. B.	Died, Dec. 21, 1920, 2 p. m.
874	1,830	829/800 L. D. control.....	Died, Dec. 21, 1920, 2 p. m.

CONCLUSIONS

The difference observed in the toxicity for chickens of the various strains of *B. botulinus* type A and type B, as well as the primary relation of *B. botulinus* type A to limber neck in poultry, suggests the following conclusions:

B. botulinus type A, via the digestive tract, is fatal to mature chickens. The symptoms induced are indistinguishable from so-called limber neck. *B. botulinus* type A has been isolated from the feed and the intestinal content of birds fatally afflicted in sporadic outbreaks of so-called limber neck. Stated another way, at least one type of limber neck occurring in poultry in Illinois is associated with *B. botulinus* type A.

B. botulinus type B, via the digestive tract, is nontoxic to mature chickens, yet the feces of chickens fed this strain are highly toxic to susceptible animals. That the virus may be distributed in nature by apparently healthy chickens or other resistant animal species is suggested.

Botulinus antitoxin type A is a specific prophylactic agent for botulism in chickens, and may be conservatively recommended in the prevention and treatment of outbreaks of so-called limber neck in poultry induced by *B. botulinus* type A.